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Controlled release of photoswitch drugs by degradable polymer microspheres

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Abstract

Background—QAQ and DENAQ are synthetic photoswitch compounds that change conformation in response to light, altering current flow through voltage-gated ion channels in neurons. These compounds are drug candidates for restoring light sensitivity in degenerative blinding diseases such as AMD.

Purpose—However, these photoswitch compounds are cleared from the eye within several days, they must be administered through repeated intravitreal injections. Therefore, we are investigating local, sustained delivery formulations to constantly replenish these molecules and have the potential to restore sight.

Methods—Here, we encapsulate QAQ and DENAQ into several molecular weights of PLGA through an emulsion technique to assess the viability of delivering the compounds in their therapeutic window over many weeks. We characterize the loading efficiency, release profile, and bioactivity of the compounds after encapsulation.

Results—A very small burst release was observed for all of the formulations with the majority being delivered over the following two months. The lowest molecular weight PLGA led to the highest loading and most linear delivery for both QAQ and DENAQ. Bioactivity was retained for both compounds across the polymers.

Conclusion—These results present encapsulation into polymers by emulsion as a viable option for controlled release of QAQ and DENAQ.

Keywords

PLGA; PLA; QAQ; DENAQ; sustained delivery; local delivery; ocular delivery; eye

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in the U.S. and third overall in the world (1). AMD leads to the loss of photoreceptors in the macula of the

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eye leading to a loss of central vision that dramatically impacts quality of life (2). Current treatment methods rely upon early diagnosis and treatment prior to retinal damage (3), but this is not always enough to stave off the disease. While the photoreceptors are lost in the macula, photoreceptors in the peripheral retina are spared. Several promising strategies have been investigated to restore visual function after the photoreceptors are lost, such as electronic retinal implants (4), gene therapy (5), and stem cell transplantation (6). All of these technologies have great promise, but they are very invasive therapies. It would be very attractive to have a therapy that could be administered in a minimally invasive way that could restore vision to the blind eye. Such a therapy would also have significant potential for blinding diseases more broadly including retinitis pigmentosa and diabetic retinopathy, two more blinding diseases resulting from the loss of rods and cones.

Photoswitch compounds offer an attractive solution. Photoswitch compounds are light activated and enable bi-directional photocontrol of neural membrane potential. The azobenzene derivatives at the center of this work function by interacting with potassium channels on cells through the tetraethylammonium bind site (7). These molecules fit into this site in their trans form but not in their cis form. The molecules undergo the transition from their trans to cis form in light-responsive manner, and the wavelengths that the molecules respond to can be tuned (8). This, then, provides a drug that can be delivered to cells to make them light sensitive and these sorts of approaches have been used in a number of fields to investigate biological functions (9, 10). However, they can do much more than being an investigational tool. They have the potential to be a therapy. (8) For example, the compound, AAQ (acrylamide-azobenzene-quaternary ammonium) has been shown to restore light sensitivity to the retina of blind mice (11). In this study, AAQ reinstated light avoidance behavior and the pupillary light reflex in mice lacking all rods and cones.

More recent molecules, QAQ (quaternary ammonium-azobenzene-quaternary ammonium) and DENAQ (diethylamine-azobenzene-quaternary ammonium), have higher light sensitivity and are even more potent. However, these compounds, like AAQ, are eliminated from the eye within hours to days after injection (12). While a short duration of action is enough for temporary photosensitization (11), many repeated injections would be required to bring about a continued effect, making a sustained delivery system attractive for therapeutic development of these compounds.

Poly(lactic-co-glycolic) acid (PLGA) and poly(lactic acid) (PLA) have strong safety records in ocular applications (13–16), and simple variations of the polymers, such as molecular weight and glucolic acid to lactic acid ratio, can alter the degradation profiles of the polymers and the subsequent release of encapsulated compounds, making them attractive materials to investigate for the sustained delivery of QAQ and DENAQ.

In this paper, we investigate the loading and delivery of QAQ and DENAQ in microspheres fabricated from varying molecular weights of PLGA as well as PLA. Here we compared loading efficiency and release profiles of QAQ and DENAQ in PLGA with a carboxyl end group (502H: M_n of ~10 kDa, 503H: M_n of ~25 kDa, and 506H: M_n of ~45 kDa) as well as PLA. The drug encapsulated microspheres released QAQ and DENAQ for over 50 days with the PLGA 502H showing the highest loading and most linear release over that time period.

The higher molecular weight polymers exhibited substantially lower loading which made the longer delivery less attractive for an injectable ocular delivery system.

2. Materials and Methods

2.1 Materials

All materials were used as received. PLGA 502H (50:50 lactic to glycolic acid ratio and an M_n of ~10 kDa) and PLGA 506H (50:50 lactic to glycolic acid ratio and an M_n of ~45 kDa) were purchased from Boehringer Ingelheim. PLGA 503H (50:50 lactic to glycolic acid ratio and an M_n of ~25 kDa) was purchased from Polysciences. The H signifies the PLGA has a carboxylic acid end group. Phosphate buffered saline (PBS) was purchased from Fisher Scientific. Dimethyl sulfoxide (DMSO), methylene chloride (DCM), deuterated chloroform, deuterated water, and poly(vinyl alcohol) (PVA) were purchased from Sigma Aldrich. QAQ and DENAQ were custom synthesized and purchased from Jubilant Chemsys, Bangalore, India) following procedures described in Banghart et al. (7).

2.2. Calibration curves

QAQ and DENAQ can be detected by ultraviolet-visible (UV-Vis) spectroscopy (Varian Cary WinUV 100 Bio UV-visible spectrophotometer). The *trans* isomer of QAQ absorbs at 375 nm and DENAQ at 455 nm. To correlate absorbance value to concentration, calibration curves were generated through serial dilution of the compounds from 100 µg/ml to 3 µg/ml in 1X PBS and in DMSO.

2.3. QAQ and DENAQ microsphere fabrication

All reactions were performed in the dark. A 20 mg/ml stock solution of each drug (QAQ or DENAQ) was made, dissolved in DMSO. Three sets of 200 mg of the polymers (PLGA 502H, PLGA 503H, or PLGA 506H) were each dissolved in 2 ml of DCM. One ml of the 20 mg/ml QAQ solution was added to one, 1 ml of the 20 mg/ml DENAQ solution was added to another, and no drug solution was added to a blank set of polymer. The polymer solutions were added to 4 ml of 5% PVA while vortexing at the maximum setting. This solution was then added dropwise to 100 ml 5% PVA spinning at 550 rpm. The solutions were stirred for 3 hours. The DENAQ-containing polymer was orange, the QAQ-containing polymer was yellow, and the blank was white.

After stirring 3 hours, the microspheres were washed to remove PVA and excess drug by centrifugation and resuspension in deionized water. The spheres were washed by centrifugation at 500 g for 5 minutes with deionized water 3 times. The spheres were then snap-frozen in liquid nitrogen and lyophilized.

2.3. QAQ and DENAQ microsphere loading

After being dried, 5 mg of the drug containing microspheres were dissolved in 1 ml DMSO and allowed to incubate for 3 hours in the dark to ensure total drug dissolution. The absorbance was taken with UV-vis spectroscopy to determine the concentration of the drug. To examine alternate initial loading concentrations, 500 µl of a 10 mg/ml stock drug solution was added to the polymer instead of 1 ml of a 20 mg/ml drug solution.

2.4. QAQ and DENAQ release

10 g of the spheres were resuspended in 1 ml of $1 \times$ PBS and placed in an incubator at 37°C . After one hour, the samples were centrifuged at 13,200 g and the supernatant was removed to analyze with UV-Vis spectroscopy. The pellet was then resuspended in 1 ml of $1 \times$ PBS and replaced into the incubator. Subsequent supernatant samples were then taken at 2 hours, 4 hours, 8 hours, 1 day, 3 days, 5 days, 1 week, and every subsequent week. The absorbance of each sample was taken by UV-Vis spectroscopy and the values compared to the calibration curve to determine the amount of drug released at the given time points.

2.6. Size characterization

Sizing of all microspheres was performed by quantifying SEM images.

2.7. Statistics

All experiments were performed in triplicate and data are presented as means $\pm$ standard deviation of the mean.

3. Results and Discussion

3.1 Calibration curves

The absorption spectra for QAQ and DENAQ were determined with UV-Vis spectroscopy. Maxima were observed at 375 nm for QAQ and 455 nm for DENAQ, as expected based on the design of these molecules (Fig. 1). One of the attractions of assaying these molecules spectroscopically is that only the active form will lead to a signal. Any loss in bioactivity would lead to a reduction in the peak (8–10).

The calibration curves of QAQ and DENAQ in both PBS and DMSO were linear for both drugs over a wide range of concentrations (Fig. 2). For the subsequent work, any samples that were believed to be more concentrated than the range tested were diluted into the linear range to accurately measure the drug concentration.

3.2. Loading study

QAQ and DENAQ were formulated with an initial 20 mg of drug to the 200 mg of polymer. For both the QAQ and DENAQ molecules, PLGA 502H led to the highest loading efficiency (Table I) with the higher molecular weight PLGAs and PLA leading to much lower loading efficiencies than the PLGA 502H. All of the PLGAs used in this study are terminated with a carboxyl group which may be interacting with the drugs and increasing loading. Since PLGA 502H has the lowest molecular weight of all of the polymers studied here, it also has the highest density of carboxyls which may account for the higher loading.

3.3 Release study

Figure 3a shows the cumulative release of QAQ from the series of polymeric microspheres over 150 days in an infinite sink model of PBS. Not surprisingly, based on the loading, PLGA 502H has the highest cumulative release. Approximately 87% of the drug ($46 \mu\text{g}$ QAQ/mg polymer) was released on the first 42 days. The release is relatively linear over this

time period no appreciable burst. As the molecular weight of the polymer is increased, the loading is reduced as noted before, but the release profiles do not change substantially. PLA microspheres, however, show a distinctly different profile with approximately 50% of the drug being released over the first three days.

Figure 3b shows the release profiles for the DENAQ spheres. DENAQ is more hydrophobic than QAQ. The same trends are present with slight variations. The PLGA 502H spheres exhibit a release of approximately 5% on day 1 in a burst release followed by a plateau and relatively linear release from day 7 to day 42 leading to delivery of 95% of the drug. Increasing the molecular weight of the polymer leads to lower overall loading as well as exaggerated delay between the initial burst and sustained release of the drug. Again, PLA leads to a burst of the majority of the drug as was seen with DENAQ. This increase in the plateau or delay before the majority of the release is consistent with other studies looking at the delivery of hydrophobic drugs (17). Overall, a relatively fast degrading polymer like PLGA 502H with the carboxyls optimizes the loading and release and would be the most attractive candidate for in vivo characterization of the controlled release of these molecules.

3.4 Size characterization

The microsphere sizes varied between approximately 5 and 35 μm (Table 2). This data is based on measurements from the SEM micrographs (Fig. 4). All of the microspheres were made using the exact same conditions, drug quantity and excipients. The only variable was the choice of polymer which clearly impacts the size. Notably, the particles with the highest loading and most linear release profiles (PLGA 502H QAQ and DENAQ spheres) also are some of the smallest spheres. As the molecular weight of the polymer increases, the spheres get larger. Again, this is consistent with other studies and may be related to the kinetics of forming particles with high molecular weight polymers (18, 19). While we did not try to section these spheres to look for pores, it is likely that the larger particles are also more porous based on previous work.

Size plays an important role in the delivery of microspheres. When injecting spheres into the eye, one can inject a much higher concentration of particles in solution when those particles are smaller. Thus, it is attractive for the purposes of in vivo work to have spheres with relatively linear release, high loading, and small diameters as is seen with the 502H formulations here.

4. Conclusion

Blinding diseases like AMD have tremendous detrimental impacts on patient health and quality of life (2, 20). For wet AMD, the form of the disease in which the vessels of the eye become leaky, anti-vascular endothelial growth factor (anti-VEGF) can halt the progression of the disease and may lead to limited visual improvement if the disease has not progressed too far. As much as drugs like the anti-VEGF compounds have impacted treatment of wet-AMD, we still lack therapies to restore vision in dry-AMD, which does not involve neovascularization, and in other blinding diseases more broadly. The photoswitch compounds offer the possibility of a drug that can restore vision, but for this to be

therapeutically viable, we need to have a delivery system that can provide local, sustained release.

Our goal in this study was to optimize the loading and delivery of QAQ or DENAQ from degradable polymer microspheres. We found retention of bioactivity after encapsulation and found a range of time frames during which QAQ and DENAQ were released. The release of QAQ and DENAQ from increasing molecular weights of the polymer results in a longer delay before burst release of the drugs. This provides a wide time range during which the drugs can be delivered. With bioactivity retention and a range of release profiles established, we believe polymer encapsulation may be well-suited for controlling the release of QAQ and DENAQ for potential therapeutic applications.

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Figure 1. Absorbance spectra of **A.** QAQ with a maximum at 375 nm and **B.** DENAQ with a maximum at 455 nm.

Figure 2.

The calibration curves of QAQ and DENAQ in various media. They are linear. **A.** The calibration curve of DENAQ in PBS. **B.** The calibration curve of QAQ in PBS. **C.** The calibration of DENAQ in DMSO. **D.** The calibration of QAQ in DMSO.

Figure 3. The cumulative release profiles of the encapsulated QAQ and DENAQ in the polymers. **A.** QAQ encapsulated in PLGA 502H, PLGA 503H, PLGA 506H, and PLA. **B.** DENAQ encapsulated in PLGA 502H, PLGA 503 H, PLGA 506H, and PLA.

Figure 4.
SEM micrographs.

Table I

The amount of drug loaded into the polymers

Microspheres	Loading (μg of drug/mg of spheres)
502H QAQ	51.15 $\pm$ 17.77
503H QAQ	8.35 $\pm$ 1.01
506H QAQ	7.06 $\pm$ 5.18
PLA QAQ	5.49 $\pm$ 0.52
502H DENAQ	62.37 $\pm$ 18.59
503H DENAQ	18.10 $\pm$ 0.65
506H DENAQ	7.55 $\pm$ 4.30
PLA DENAQ	2.45 $\pm$ 0.31

Table II

Sizing of the polymer microspheres as measured by SEM

Microspheres	Mean diameter (μm)
502H QAQ	11.7 $\pm$ 7.93
503H QAQ	10.61 $\pm$ 7.04
506H QAQ	19.12 $\pm$ 117.11
PLA QAQ	10.25 $\pm$ 5.22
502H DENAQ	5.45 $\pm$ 2.44
503H DENAQ	33.87 $\pm$ 18.72
506H DENAQ	13.72 $\pm$ 7.87
PLA DENAQ	6.34 $\pm$ 3.09